iv) checking for cytotoxicity of the expressed recombinant PA-I on mammalian cells in vitro,

v) assaying the inhibitory action of recombinant PA to inhibit pore-forming ability of native PA in vitro at a concentration which is equimolar or lower than that of native PA, and

vi) in vivo evaluation of the mutant protein for inhibitory action on anthrax toxin activity in equimolar ratio with native PA plus lethal factor (LF).

REMARKS

Claim 11 has been replaced with replacement claim 11 to correct for multiple dependencies. No new matter has been entered. Claims 1-11 are in this application.

A prompt and favorable action on the merits is earnestly solicited. It is believed that no fee is required. The Commissioner is authorized to charge any deficiency or credit any overpayment to Deposit Account No. 13-2165.

Respectfully submitted,

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DATE: March 29, 2001

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Marked-Up Version:

11. A method for developing the novel recombinant anthrax toxin inhibitor protein as claimed in claim 1, said method comprising steps of:

i) mutagenesis using the PCR primers [of claim 3] :

Primer 1:

5'< ATT ACT AAA TCC TGC AGA TGT AGT GAT ATT ACC AGT AAA GCC GTT CTG ATA CCC TGC TGA AAT TGA AAC TCC TAC AGT ATT AGC ATC CCT ACT TGT AGA AGT ATT TTT AC >3'

Primer 2:

5'< GT GAT TAA TAA AGC TTC TAA TTC >3'

ii) cloning the amplified mutated fragment into the appropriate vector and inserting it into an appropriate host for expression of the mutated gene,

iii) purifying the mutant protein followed by characterization of the expressed mutant protein,

iv) checking for cytotoxicity of the expressed recombinant PA-I on mammalian cells in vitro,

v) assaying the inhibitory action of recombinant PA to inhibit pore-forming ability of native PA in vitro at a concentration which is equimolar or lower than that of native PA, and

vi) in vivo evaluation of the mutant protein for inhibitory action on anthrax toxin activity in equimolar ratio with native PA plus lethal factor (LF).